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The inhibitory effect of a non-yessotoxin-producing dinoflagellate, *Lingulodinium polyedrum* (Stein) Dodge, towards *Vibrio vulnificus* and *Staphylococcus aureus*

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Abstract: The increased bacterial resistance to antibiotics has caused global concern, prompting the search for new compounds. Because of their abundance and diversity, marine phytoplankton are an important potential source of such compounds. Research on dinoflagellates has led to the discovery of inhibitors of bacterial growth. The marine dinoflagellate *Lingulodinium polyedrum* blooms in different regions of the world, including Mexico, and is also known to regulate the growth of other species in coastal waters. Here, we investigated the taxonomy of this dinoflagellate and characterized the ability of its extracts to inhibit the growth of two bacteria of medical importance (*Vibrio vulnificus* and *Staphylococcus aureus*). Taxonomic characterization was performed by PCR and gene amplification of ITS, and confirmed that the species isolated off the Pacific coast of Mexico was *L. polyedrum*. To prove the inhibitory effect of *L. polyedrum* extracts, cultures were harvested by centrifugation. Pellets from three cellular abundances were extracted with water, methanol, hexane and chloroform. The experiments on *V. vulnificus* showed a high growth inhibition for the four extracts, ranging from 77 to 98 %. Surprisingly, the growth inhibition was lower when the extracts originated from a higher *L. polyedrum* cell abundance, ranging from 0 to 34 %. For *S. aureus*, the growth inhibition was also high, but not statistically different for all extracts and cell abundances, ranging from 62 to 99 %. This study obtained promising results for future pharmacological applications. Our Mexican strain of *L. polyedrum* did not produce any detectable yessotoxins. Rev. Biol. Trop. 64 (2): 805-816. Epub 2016 June 01.

Palabras clave: proliferaciones algales, antibiótico, resistencia bacteriana, *Lingulodinium polyedrum*, fitoplancton, *Staphylococcus aureus*, *Vibrio vulnificus*, yessotoxina.

Since the discovery of antibiotics in the early 1940s, their clinical use has resulted in greatly improved health care. Human deaths from bacterial infections have been reduced and life expectancy has increased. After the emergence of antibiotic resistance in the 1970s, research has been focused principally on modifications to semisynthetic compounds that were already clinically proven (Stach, 2010). Currently, the resistance of pathogenic bacteria to multiple drugs is a global problem, causing increased concern in health care institutions (Klevens et al., 2007; Fischbach & Walsh, 2009). One possible solution is to discover and introduce new antibacterial medications. Marine eukaryotic microalgae are a potential

source of these new compounds; they offer a high genetic diversity and constitute an untapped resource of novel natural products. Their ability to withstand environmental stress and outcompete other marine organisms is related to their capacity to produce a vast array of secondary metabolites, which have considerable value in the biotechnology, aquaculture, health and food industries (Anderson, 1996). Likewise, several studies have reported antibacterial activity in the cell lysates or extracts of various microalgal species (Desbois et al., 2009; Blunt et al., 2011; de Jesus Raposo et al., 2013).

Dinoflagellates are a large group of flagellate protists, belonging mostly to marine phytoplankton, some of which are known to form harmful algal blooms that are an important source of marine biotoxins (Tomas, 1997; Gallardo-Rodríguez et al., 2012). Bioactive compounds from dinoflagellates have received increased attention because of their impact on the safety of seafood and their potential uses in biomedical, toxicological and pharmacological research. Despite the many interesting bioactive compounds isolated from dinoflagellates (Konishi et al., 2004; Camacho et al., 2007; Blunt et al., 2011), only a few have led to commercial products. This is partly due to the limited amount of dinoflagellate toxin that can be amassed for detailed clinical evaluation (Gallardo-Rodríguez et al., 2012). Nevertheless, recent patents and patent applications related to dinoflagellate toxins have been published (Selander & Pavia, 2008; Paul, 2011). One of the few examples of medical uses is the dinoflagellate toxin, gonyautoxin (Garrido et al., 2005). *Lingulodinium polyedrum* (Stein) Dodge is a dinoflagellate that blooms in coastal waters of Colima, Mexico (Quijano-Scheggia et al., 2013). It is easy to isolate and maintain in culture. Here, we probe its potential as source of antibacterial compounds against two bacteria of medical importance: *Vibrio vulnificus* and *Staphylococcus aureus*. *Vibrio vulnificus* is a Gram-negative, oxidase-positive, facultative anaerobe. In warm coastal waters, it causes vomiting, diarrhea and abdominal pain, when raw or undercooked shellfish, especially oysters, are consumed (Strom & Paranjpye, 2000; Tortora Funke & Case, 2007; Horseman & Surani, 2011). It has become resistant to various antibiotics (Kim et al., 2011; Shaw et al., 2014). *Staphylococcus aureus* is a Gram-positive facultative anaerobe, which is widely distributed and capable of producing a broad range of diseases, from skin infections to life-threatening illnesses. It, also, has become resistant to several antibiotics (Lowy, 2003), resulting, for example, in Methicillin-resistant *Staphylococcus aureus* (MRSA) infection (Appelbaum, 2007).

The present study was carried out to discover if cell extracts of *L. polyedrum* may contain antibacterial compound(s) that may inhibit the growth of the pathogenic bacteria *V. vulnificus* and *S. aureus*.

MATERIALS AND METHODS

Sampling and culturing: Phytoplankton samples were collected with a plastic bottle during a bloom of *L. polyedrum* in May 2012, off the coast of Manzanillo, Colima, Mexico (19°7'3.00" N - 104°22'23.52" W), and maintained at 4 °C. Single cells were isolated under a Motic AE31 inverted microscope (Ted Pella, Inc., Redding, California, USA) with a glass Pasteur pipette. They were then transferred to tissue culture plates (Assay Plate 96 Well Flat Bottom, Corning, NY, USA) containing 0.4 mL of L1 medium (Guillard, 1975) at a salinity of 30, modified by addition of 10^{-8} M H_2SeO_3 and by reducing the concentration of $CuSO_4$ to 10^{-8} M (Band-Schmidt et al., 2005). After reaching a density of 10^4 cells/L, the non-axenic cultures were transferred successively to 50, 250 and 1 000-mL flasks. All cultures were maintained at 21 °C under cool-white fluorescent tubes (Phillips F96T12/TL865/EW, 60 W, USA) with an irradiance of 90 µmol photons/m^2.s and a 12:12 h light:dark photoperiod. Cell abundance was determined at 200-400 x magnification using a Motic AE31 inverted bright-field light microscope, according to Utermöhl (1931) and Throndsen (1995).

Gene amplification and sequencing: Cells were harvested during the late exponential growth phase by centrifugation at 2 000 ×g for 10 min, with a MAX RCT centrifuge (REXMED, Kaohsiung, Taiwan). The pellets were frozen at -20 °C and the DNA was extracted following Lundholm, Moestrup, Hasle & Hoef-Emden (2003). The Internal Transcribed Spacer (ITS1-5.8S-ITS2) region of the rDNA was amplified and sequenced using the primers ITS1 and ITS4 (White Bruns, Lee & Taylor, 1990).

The ITS rDNA sequences were aligned using ClustalW (Thompson, Higgins, & Gibbson, 1994). The alignment included 593 positions. Separate analyses of ITS2 were also performed as in Koetschan et al. (2010). The ITS analyses were rooted with seven outgroup taxa (Table 1).

Neighbor-Joining (NJ) and Maximum-Likelihood (ML) were performed using MEGA 8 (Kumar, Tamura, & Nei, 2004). Bayesian analyses were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), using four chains of 1 000 generation each. The

TABLE 1
List of the species and strains used in the present study, including *Lingulodinium polyedrum* from Manzanillo, Mexico (in bold)

Species	Location	GenBank accession
L. polyedrum	Italy	AM184208
L. polyedrum	United Kingdom	EU177126
L. polyedrum	United Kingdom	EU177127
L. polyedrum	United Kingdom	EU177128
L. polyedrum	California, USA	EU532480
L. polyedrum	California, USA	EU532481
L. polyedrum	California, USA	EU532482
L. polyedrum	British Columbia, Canada	FJ823575
L. polyedrum	British Columbia, Canada	FJ823576
L. polyedrum	British Columbia, Canada	FJ823577
L. polyedrum	La Paz, Mexico	JQ616824
L. polyedrum	Manzanillo, Mexico	KR185944
Gonyaulax polyedra	Korea	AF377944
Akashiwo sanguinea	Korea	AY831412
Akashiwo sanguinea	Korea	AY831411
Alexandrium peruvianum	Rhode Island, USA	JX113683
Dinophysis caudata	Maryland, USA	EU780642
Dinophysis caudata	France	AY040584
Gymnodinium catenatum	Korea	DQ779989
Gymnodinium catenatum	Korea	DQ779990
Gyrodinium instriatum	Iran	JN020162
Heterocapsa triquetra	Korea	HQ902267
Karenia brevis	British Columbia, Canada	FJ823563
Karenia brevis	British Columbia, Canada	FJ823562
Oxyrrhis marina	United Kingdom	FJ853681
Oxyrrhis marina	United Kingdom	GQ487326
Prorocentrum micans	Maryland, USA	EU780638
Prorocentrum micans	New Brunswick, Canada	EU927533
Scrippsiella trochoidea	China	JQ250798
Scrippsiella trochoidea	Germany	HQ729501

temperature was set to 0.25. Sample frequency was 500, and the number of burn-in generations was 5 000.

The secondary structure of ITS2 was predicted using RNAstructure ver. 5.7 (Mathews et al., 2004). Our strain of *L. polyedrum* from Manzanillo (Table 1) was used as a template for homologous modeling of other *L. polyedrum* strains through ITS2 Database (http://its2-old.bioapps.biozentrum.uni-wuerzburg.de/cgi-bin/index.pl?custom) (Schultz et al., 2006; Selig, Wolf, Mülle, Dandekar, & Schultz, 2008; Koetschan et al., 2010). Helices were recognized by comparing the ITS2 regions of several *L. polyedrum* sequences obtained from the above ITS2 database. The helices were named according to Mai & Coleman (1997). Secondary structures of *L. polyedrum* ITS2 were illustrated by VARNA (Darty Denise & Ponty, 2009).

Crude extracts: Exponential-phase *L. polyedrum* cells were harvested by centrifugation for 10 min each at 2 500 × g and then at 20 000 × g, at 4 °C, with the same centrifuge as above. Three cell abundances of *L. polyedrum* were used for each test of antibacterial activity (see below). The pellets were lyophilized using a FreeZone 6 lyophilizer (Labconco, Missouri, USA). Four separate extractions were performed on the pellets by adding 1.5 mL of water, methanol, hexane or chloroform for 10 min. Silicon pearls were added to each solvent and then cells were disrupted using a Mini-BeadBeater (Biospec, Oklahoma, USA) for 10 s. Each supernatant was then placed in an Eppendorf tube and allowed to evaporate at room temperature. The extracts were stored in the evaporated state at 4 °C and then resuspended in 500 µL of sterile distilled water just prior to use.

Experiments to test for antibacterial activity: To determine if there was antibacterial activity in the compounds extracted from *L. polyedrum*, two bacteria were used: *Vibrio vulnificus* (ATCC 27562, Gram-negative) and *Staphylococcus aureus* (ATCC 29213, Gram-positive). The former was incubated in tryptone soy broth and the latter in Brain Heart Infusion (BHI) broth, each for 24 h at 35-37 °C. The bacterial abundance in the broth was standardized to 0.05 absorbance units (at 400 nm) with a Jenway 6 500 spectrophotometer (Staffordshire, UK). One µL of each broth was then diluted into 999 µL of Phosphate Buffered Saline (PBS) and labeled as standardized bacterial dilution (SBD).

Inhibition experiments were carried out in triplicate by adding 10 µL of SBD to 10, 25 and 50 µL of each extract and completing to 100 µL with PBS. As a control, 10 µL of SBD was added to 90 µL of distilled water. Each tube was incubated for 2 h at 35-36 °C, after which three drops of each solution were placed onto single Petri plates made with tryptone soy agar for *V. vulnificus* and BHI agar for *S. aureus*. As a control, three drops of SBD solution were placed onto another Petri plate. The Petri plates were incubated for 24 h at 35-37 °C. Cell colonies were counted in the control plates and compared to those numbers in each cell extract and cell abundance treatment, and the percent inhibition for each treatment was then calculated. The control ranged from 15 to 30 Colony Forming Units (CFU). The Student's t-test was used to determine whether the results were statistically significant.

Toxin extraction and analysis: The *L. polyedrum* extracts were examined for the possible presence of yessotoxins (YTXs) and some analogs and derivatives (Table 2), as this dinoflagellate is a known producer of YTXs (Paz et al., 2008). YTXs were extracted from 50 mL of late-exponential phase *L. polyedrum* culture, centrifuged for 10 min each at 2 500 × g and then at 20 000 × g, as above. The pellets were extracted with 10 mL of methanol and then frozen at -20 °C prior to being sent to the Centro de Investigación Médica Aplicada (CIMA; Galicia, Spain) for further processing, as follows. The extract was concentrated in a Thermo SpeedVac rotary evaporator and filtered through a Macherey-Nagel 0.22-µm PES syringe filter. A 10-µL aliquot was then injected

TABLE 2
Main mass spectrometry parameters used in the screening and quantification of yessotoxin (YTX) and its derivatives; all were below the limit of detection

Toxin	Transition
41-ketoYTX	538.4>396.4
41-ketoYTX	538.4>467.4
TrinorYTX	550.4>396.4
TrinorYTX	550.4>467.4
Trinor-Homo-YTX	557.4>403.4
Trinor-Homo-YTX	557.4>474.4
44-oxotrinorYTX	558.4>404.4
44-oxotrinorYTX	558.4>475.4
44,55-diOH YTX	565.4>396.4
41a-homo-44oxotrinorYTX	565.4>411.4
44,55-diOH YTX	565.4>467.4
41a-homo-44oxotrinorYTX	565.4>482.4
YTX	570.4>396.4
YTX	570.4>467.4
Homo-YTX	577.4>403.4
Homo-YTX	577.4>474.4
45-OH-YTX	578.4>396.4
45-OH-YTX	578.4>467.4
45-OH-Homo-YTX	585.4>403.4
45-OH-Homo-YTX	585.4>474.4
COOH-YTX	586.4>396.4
COOH-YTX	586.4>467.4
COOH-Homo-YTX	593.4>403.4
COOH-Homo-YTX	593.4>474.4
COOH-45-OH-YTX	594.4>396.4
44,55-diOH-41ahomoYTX	594.4>403.4
COOH-45-OH-YTX	594.4>467.4
44,55-diOH-41ahomoYTX	594.4>474.4
32-O-monoglycosyl-YTX	636.4>462.4
32-O-monoglycosyl-YTX	636.4>533.4
32-O-diglycosyl-YTX	702.4>528.4
32-O-diglycosyl-YTX	702.4>599.4

into a LC-MS/MS system composed of a Thermo TSQ Quantum Access Max coupled to an Accela UHPLC system through an HESI-II electrospray interface. The method of Regueiro Martín-Morales, Álvarez & Blanco (2011) was used, but modified by the use of a 50-mm Gemini NX C18 3 µm, 2.1 × 50 mm column (Phenomenex), and a different elution gradient, as detailed below. The chromatographic phases were 6.7 mM ammonium hydroxide (phase A) and acetonitrile in 6.7 mM ammonium hydroxide (9:1, v:v) (phase B). For the on-line solid-phase extraction (SPE), a Security Guard with a Gemini NX C18 4 × 2 mm cartridge (Phenomenex), and a mixture of phases A and B (90:10, v/v) as loading phase, were used.

The chromatographic conditions started at 90 % phase A, held until min 1.50, after which the flow through the SPE column was diverted to the chromatographic column. The percentage of this phase was then reduced to 20 %, 15 % and 5 % at min 3.85, 4.00 and 4.75, respectively, before being held at 5 % for two additional min. Finally, the conditions were reverted to the initial proportion in order to equilibrate the column for the next injection. The mass spectrometer conditions were as follows: ionization mode: negative spray; voltage: 3 000 V; sheet gas: 40 (nominal); auxiliary gas 10 (nominal); vaporizer temp: 105 ºC; capillary temp: 360 ºC; collision cell gas pressure: 1.5 Torr; collision energy: 30. The transitions used to identify YTXs are given in Table 2. The limit of detection was 0.13 ng/mL of extract.

RESULTS

Morphology: The clonal culture of *Lingulodinium polyedrum* from Manzanillo was morphologically similar to the original description (Kofoid, 1911). Cells showed a polyedral shape, ranging from 35.6-47.9 µm in length and 30.9-38.4 µm in width (n=21). Antapical spines and an apical horn were absent. The theca is thick, well defined and areolate, with pores in the depressions. Plate formula: Po, 3', 3a, 6", 6c, 7s, 6''' and 2'''' (Fig. 1).

Phylogenetic inference: The phylogenetic analysis using ITS nucleotide sequences yielded similar tree topologies by NJ, ML and BI. Aligned sequences produced a total of 593 characters (including gaps), of which 489 characters were constant, and 218 variable characters were parsimony informative. Our strain of *L. polyedrum* from Manzanillo clustered with other strains from different regions of the world in a moderate to strongly supported clade,

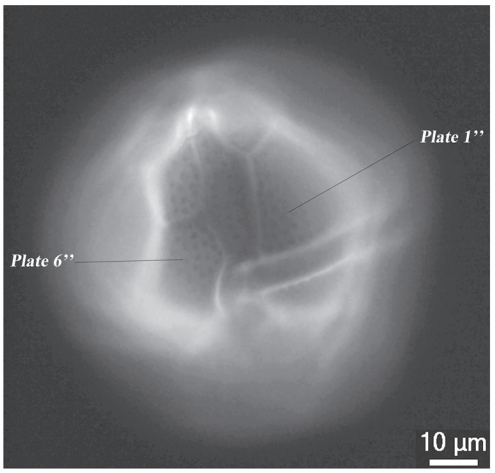

Fig. 1. Ventral views of *Lingulodinium polyedrum* from Manzanillo. Light microscopy stained with calcofluor white.

giving bootstraps values of 99 %, 64 % and 96 %, for NJ, ML and BI, respectively (Fig. 2).

In general, the ITS2 transcript of *L. polyedrum* showed four universal helices. The secondary structure of ITS2 of *L. polyedrum* from Manzanillo showed some genetic divergence with strains from America, Europe and Asia, giving eight Single Nucleotide Polymorphisms (SNPs), four Hemi-Compensatory Base Change (HCBCs) and one deletion (Fig. 3).

Antibacterial activity: The experiment on *V. vulnificus*, using an aqueous extract from *L. polyedrum* with an abundance of 2×10^6 and 5×10^6 *L. polyedrum* cells, showed a growth inhibition of 96.4 % and 93.6 %, respectively (Fig. 4). For extracts in the other solvents, the

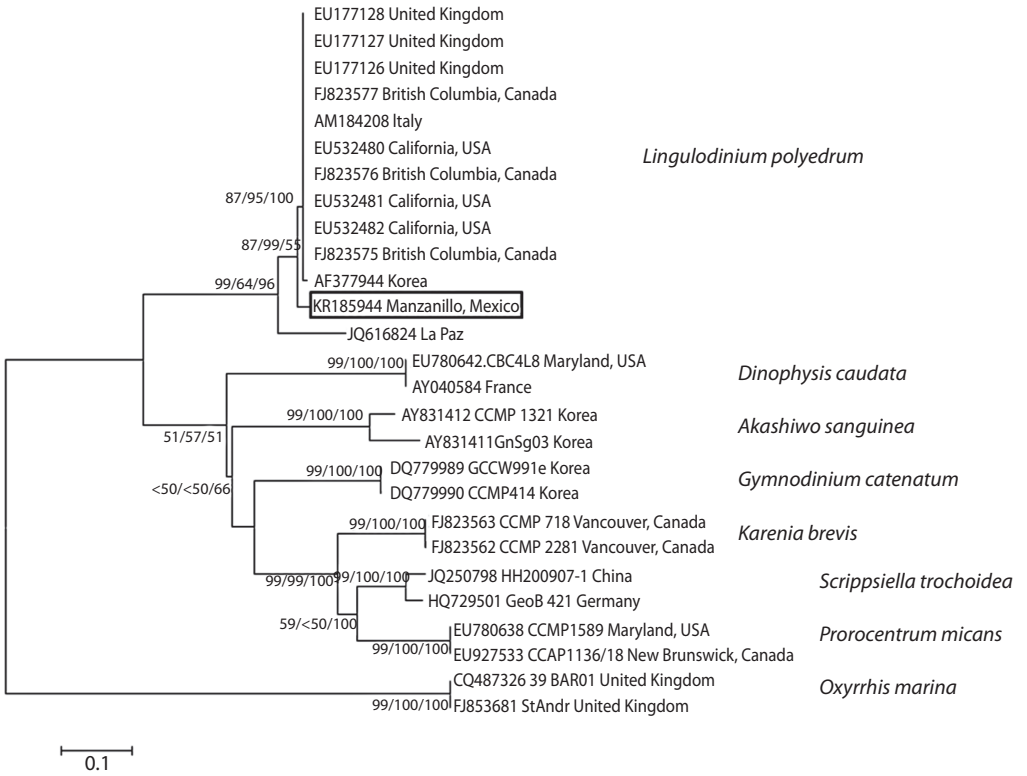

Fig. 2. Neighbor-joining (NJ) phylogenetic tree of *Lingulodinium polyedrum* based on ITS1, 5.8S, and ITS2 sequences. Bootstrap values from NJ, maximum likelihood (ML) and Bayesian are shown. Only bootstrap values > 50 % are indicated. Sequence in box indicates strain obtained for the present study (from Manzanillo, Mexico); sequences of the other strains were obtained from GenBank.

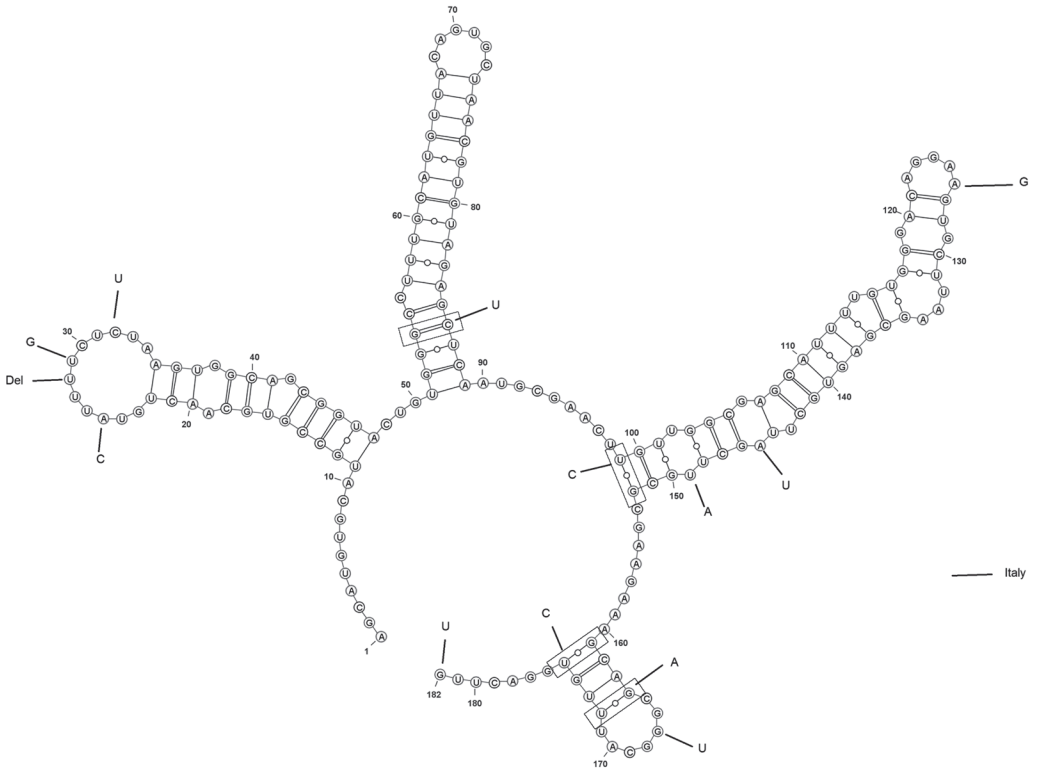

Fig. 3. Secondary structures of ITS2 from *Lingulodinium polyedrum* from Manzanillo, Mexico. Rectangles indicate hemicompensatory base changes (HCBCs).

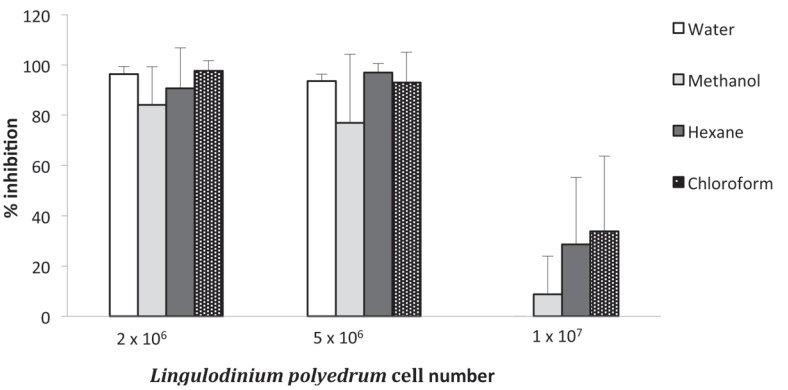

Fig. 4. Percent growth inhibition of *Vibrio vulnificus* with four extracts and three cell abundances of *Lingulodinium polyedrum*.

inhibitions were: 84.1 % and 77.0 % (methanol); 90.7 % and 97.0 % (hexane); and 97.7 % and 93.0 % (chloroform), for the two cell abundances, respectively. Using a higher abundance of 1×10^7 cells, the inhibition was lower: 8.8 % (methanol); 28.6 % (hexane); 33.8 % (chloroform); no inhibition was observed for the aqueous extract (Fig. 4).

The growth inhibition of *S. aureus* was maximal for abundances of 6.48 × 10^4 and 1.26 × 10^5 *L. polyedrum* cells for each extract, as follows: 97.6 % and 90.8 % (aqueous); 95.4 % and 98.2 % (methanol); 98.1 % and 99 % (hexane), for the two cell abundances, respectively (Fig. 5). For the chloroform extract, the inhibition was 97.5 % for a cell abundance of 6.48 × 10^4, and 98.8 % for 1.26 × 10^5 cells. The growth inhibition for a cell abundance of 3.24 × 10^5 was lower, ranging from 62.1 % (aqueous) to 69.9 % (hexane) (Fig. 5).

There were significant differences in the growth inhibition of *V. vulnificus* between the different extracts in each group with the 2 × 10^6 and 1 × 10^7 cell abundances (Table 3). With the 2 × 10^6 and 5 × 10^6 cell abundances, the difference was significant only for the aqueous

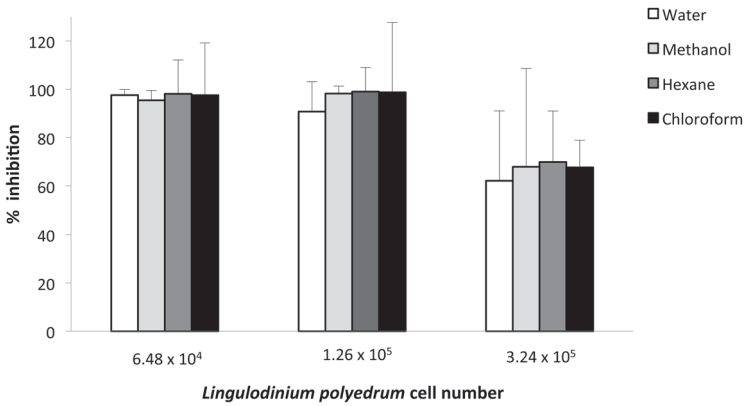

Fig. 5. Percent growth inhibition of *Staphylococcus aureus* with four extracts and three cell abundances of *Lingulodinium polyedrum*.

TABLE 3
Student's t-test analysis of the growth inhibition of *Vibrio vulnificus*, with three cell abundances and four extraction solvents

		2 x 10^6 cells				5 x 10^6 cells				1 x 10^7 cells			
		Water	Methanol	Hexane	Chloroform	Water	Methanol	Hexane	Chloroform	Water	Methanol	Hexane	Chloroform
2 x 10^6 cells	Water					x				x	x	x	x
	Methanol					x				x	x	x	
	Hexane					x				x	x	x	x
	Chloroform					x				x	x	x	x
5 x 10^6 cells	Water						x	x	x	x			
	Methanol									x	x		
	Hexane									x	x	x	x
	Chloroform									x	x	x	x
1 x 10^7 cells	Water												
	Methanol												
	Hexane												
	Chloroform												

x = significantly different ($p < 0.05$).

extract. For the cells abundances of 1×10^7 and 5×10^6, there were significant differences for most combinations of the different extracts, with the exceptions of the following combinations: water-methanol, hexane-chloroform, and methanol-hexane-chloroform. Results for the experiments with *S. aureus* did not show any significant differences in growth inhibition, for any of the four extracts and three cell concentrations.

Toxin analysis: No YTXs, nor analogs or derivatives, were detected in the *L. polyedrum* isolates from Manzanillo.

DISCUSSION

Marine microorganisms remain an abundant source of novel and biologically active metabolites, with 273 new compounds reported up to 2009 (Blunt et al., 2011). Thus, dinoflagellates can also provide numerous and potentially useful bioactive products, although relatively few have been investigated because of a limited supply from nature (Gallardo-Rodríguez et al., 2012).

Our study shows that the strain isolated from Manzanillo, off the Pacific coast of Mexico, belongs to *Lingulodinium polyedrum*. This species has been described in Mexican Pacific waters (Morquecho & Lechuga-Devéze, 2004; Peña-Manjarrez, Gaxiola-Castro, & Helenes-Escamilla, 2009; Ruiz-de la Torre, Ochoa, & Almeda-Jauregui, 2013) as well as worldwide, including upwelling systems (Smayda & Trainer, 2010). The species identity was confirmed by morphological as well as phylogenetic characterizations. The analysis of the secondary structure of ITS2 showed eight SNPs, four HCBCs and one deletion. Our *L. polyedrum* strain from Manzanillo did not produce any detectable YTXs or analogues. Other non-toxic strains of *L. polyedrum* from Norway, Spain and California (USA) have also been reported (Armstrong & Kudela, 2006). In contrast, toxic strains have been reported from Italy, the United Kingdom, Ireland, Galicia and Andalucía (Spain) (Paz, Riobó, Fernández, Fraga, & Franco, 2004; Paz et al., 2008) and California (USA) (Armstrong & Kudela, 2006).

We confirmed that this non-axenic *L. polyedrum* strain inhibits the growth of *V. vulnificus* and *S. aureus*. The inhibition of *V. vulnificus* growth by extracts in the four solvents was high, with no statistical differences between the *L. polyedrum* cell abundances of 2×10^6 and 5×10^6, except for the aqueous extract. In contrast, the inhibition was lower when higher cell abundance (1×10^7) was used, and no inhibition was observed for the aqueous extract. We hypothesize that the bioactive compounds trigger the growth inhibition at low concentrations because the compounds can penetrate the cell wall. At higher concentrations, however, they may saturate the receptors on the cell wall, thus not penetrating the cell and allowing bacterial growth. The production of inhibitory compounds may also be higher at low cell abundances. For example, Pérez, Band, Ortíz & Sobrino. (2014) found that less toxic compounds were generated at higher cell concentrations of *Chattonella* spp. (Raphidophyceae).

Our results showed that the growth of *S. aureus* is also inhibited by extracts from the four solvents and three cell abundances of *L. polyedrum*, with no statistically significant differences found among these treatments. Likewise, methanolic extracts from the diatom *Chaetoceros muelleri* showed effective inhibition of *S. aureus* growth (del Pilar Sánchez, Licea & Bernáldez 2010). The antibacterial activity from this diatom species has been associated with several fatty acids, which induced lysis in bacterial protoplasts by disrupting the cell membrane. These compounds can penetrate the meshwork of peptidoglycan polymers in the cell wall and reach the bacterial membrane, which leads to the disintegration of Enterobacteriaceae such as *E. coli* (Shanmugapriya & Ramanathan, 2011).

These results showed that *L. polyedrum* contains useful secondary metabolites against *V. vulnificus* and *S. aureus*, and that it could be a source of biologically active compounds with potential application in the pharmaceutical industry. Although many compounds isolated

from dinoflagellates have shown bioactivities of interest (Camacho et al., 2007; Blunt et al., 2011), only a few have led to commercial products (Gallardo-Rodríguez et al., 2012).

The morphological and molecular characterization of the isolate from Manzanillo, on the Mexican west coast, confirmed it as *L. polyedrum*. Cell extracts from this dinoflagellate showed a high percentage of growth inhibition against both a Gram-negative (*V. vulnificus*) and a Gram-positive (*S. aureus*) pathogenic bacterium. This is the first phase in the quest to obtain antimicrobial agents with possible pharmaceutical uses from *L. polyedrum*. It is now necessary to identify and evaluate the antibacterial activity of individual compounds, a step that is underway.

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RESUMEN

El aumento de la resistencia bacteriana a los antibióticos ha causado preocupación a nivel mundial, por lo que se ha promovido la búsqueda de nuevos compuestos. Debido a su abundancia y diversidad, el fitoplancton marino constituye una importante fuente potencial de tales compuestos. La investigación sobre dinoflagelados ha llevado al descubrimiento de inhibidores de crecimiento bacteriano. El dinoflagelado marino *Lingulodinium polyedrum* causa proliferaciones algales en diferentes regiones del mundo, incluyendo México, y también se sabe que regula el crecimiento de otras especies en las aguas costeras. En este trabajo, se investiga la taxonomía de este dinoflagelado y se caracteriza la capacidad de sus extractos para inhibir el crecimiento de dos bacterias de importancia médica (*Vibrio vulnificus* y *Staphylococcus aureus*) en placas de cultivo de agar. La caracterización taxonómica se realizó por PCR y amplificación del gen de ITS, y se confirmó que la especie aislada en la costa del Pacífico de México fue *L. polyedrum*. Para demostrar el efecto inhibidor de los extractos de *L. polyedrum*, los cultivos se cosecharon por centrifugación. Los pellets de tres abundancias celulares se extrajeron con agua, metanol, hexano y cloroformo. Los experimentos en *V. vulnificus* mostraron una inhibición alta del crecimiento para los cuatro extractos, variando entre 77 y 98 %. Sorprendentemente, la inhibición del crecimiento fue menor cuando los extractos se originaron a partir de una mayor abundancia de células *L. polyedrum*, varía de 0 a 34 %. Para *S. aureus*, la inhibición del crecimiento también fue alta, pero no estadísticamente diferente para todos los extractos y abundancias de células, con un rango de 62 hasta 99 %. Esto resultados son prometedores para futuras aplicaciones farmacológicas. La cepa mexicana de *L. polyedrum* no produjo yesotoxinas detectables.

Palabras clave: proliferación de algas, antibioticos, resistaencia bacteriana, *Lingulodinium polyedrum*, fitoplancton, *Staphylococcus aureus*, *Vibrio vulnificus*, yesotoxinas.

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